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(71) Applicant *(for all designated States except US)*: PHARMA PACIFIC PTY. LTD. [AU/AU]; Suite 6002, Bayside Plaza, 376 Bay Street, Brighton–Le–Sands, NSW 2216 (AU).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: TOVEY, Michael, Gerard [GB/FR]; 7, rue la Grange, F–75005 Paris (FR).

(74) Agents: IRVINE, Jonquil, Claire et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).

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(54) Title: THERAPEUTIC TREATMENT WITH IMMUNOGENIC DRUGS

(57) Abstract

The invention provides a kit for the therapeutic treatment of a subject with an immunogenic drug, which kit comprises (a) a first formulation comprising a compound in a dosage form for administration by an oromucosal route to induce immunological tolerance to the drug, and (b) a second formulation comprising the drug in a dosage form for administration to effect the therapeutic treatment. The administration of the first formulation enhances the effectiveness of the second formulation by reducing the immune response to the drug in the second formulation. The drug may be a peptide drug, such as an interferon, a cytokine, a growth factor, a soluble receptor or an antibody.

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THERAPEUTIC TREATMENT WITH IMMUNOGENIC DRUGS

The invention relates to therapeutic treatment with immunogenic drugs such as peptide drugs, including proteins.

BACKGROUND TO THE INVENTION

Systemic administration of an immunogenic drug such as a peptide drug elicits antibodies against the drug. This limits the effectiveness of the drug, particularly if injected repeatedly into a patient over a period of time. Examples of such drugs include interferons (e.g. alpha, beta and gamma), interleukins (e.g. IL-2, IL-4, IL-10, IL-12, IL-15), growth factors (e.g. EPO, G-CSF, GM-CSF), monoclonal antibodies (e.g.OKT3, anti-TNF alpha, anti-IL2 R alpha, anti-IFN alpha R1), and soluble receptors (e.g. TNF alpha SR p75 - Fc IgG1, IL-1 SR, IFN alpha SR1).

Humanisation of mouse or rat monoclonal antibodies has been used as a means of rendering such antibodies less immunogenic in the hope of preventing the production by the patient of antibodies directed against the injected antibody, e.g. anti-IgG or anti-idiotype antibodies. Unfortunately humanisation of monoclonal antibodies, including CDR grafting, does not prevent the production of anti-idiotypic antibodies but merely slows down the process. Furthermore, humanised antibodies are in certain cases less effective than the original mouse antibody. Even in the case of recombinant forms of authentic human proteins such as interferon α2 as many as 80 % of individuals treated with such molecules have been reported to produce antibodies against the drug which can reduce its effectiveness (Schellekens et al., 1997, J. Interferon & Cytokine Res. 17:suppl. 1, S5-S8).

Oral administration of an antigen at appropriate dosage is known to induce "immunological tolerance", i.e suppression of the immune response to a subsequent systemic challenge by the antigen. Feeding of the antigen elicits a local immune response in the intestinal mucosa, but responses to the same antigen given

subsequently by a systemic route are suppressed.

This phenomenon has been exploited in the treatment of autoimmune diseases, i.e. diseases in which a subject's immune system attacks its own tissues. Such diseases include multiple sclerosis, rheumatoid arthritis, insulin-dependent diabetes, psoriasis and lupus erythematosus.

A number of studies have shown that oral administration of a protein such as myelin basic protein, a component of the myelin sheath of nerves, or Type II collagen, a constituent of cartilage, can induce tolerance to such proteins that are recognised as self-antigens by autoreactive T-cells in patients with multiple sclerosis and rheumatoid arthritis respectively. A number of clinical trials are currently being conducted in which patients with multiple sclerosis and rheumatoid arthritis are treated with drugs based on oral formulations of native human peptides.

It is known that there are at least three levels of oral tolerance, namely "active suppression", "clonal anergy" and "clonal deletion". Active suppression is induced by oral administration of a relatively low dose of antigen and leads to immunological tolerance through inhibition of lymphocyte function. Clonal anergy is induced by oral administration of a higher dose of antigen and leads to lymphocytes that are able to recognise antigen but not respond. Clonal deletion is induced by oral administration of a very large dose of antigen and leads to elimination of reactive lymphocytes.

Although active suppression was considered to be mediated by the induction of regulatory T-cells in the gut-associated lymphoid tissue (GALT) which then migrate to the systemic immune system, recent evidence suggests that one of the primary mechanisms of active cellular suppression is via the secretion of suppressive cytokines including the Th2 cytokines IL-4, IL-10, and the Th3 cytokine transforming growth factor β (TGF-β), following antigen specific triggering (Chen et al. 1994, Science, 265, 1237-1240). Oral administration of IL-4 and, IL-10 has been

shown to potentiate oral tolerance (Weiner, H.L., 1997, Immunology Today 18, 335-343). Thus oral tolerance can be broadly defined as the inhibition of a Th1 response in the periphery, either by large doses of antigen which induce anergy and/or clonal deletion, or by gut-induced Th2 or Th3 type regulatory cells.

SUMMARY OF THE INVENTION

The invention stems from a hypothesis of the inventor that oral administration of an immunogenic drug or a related compound may induce immunological tolerance to the drug, so that when the drug is subsequently administered its pharmacokinetics and/or clinical effectiveness are improved. This approach can be used in the therapeutic treatment of a subject with an immunogenic drug by

(a) administering to the subject by an oromucosal route a first formulation comprising a compound in a dosage form which induces immunological tolerance to the drug, and

(b) administering to the subject a second formulation comprising the drug in a dosage form that effects the therapeutic treatment.

The administration of the first formulation enhances the pharmacokinetics and/or effectiveness of the second formulation by inducing immunological tolerance and reducing the immune response to the drug in the second formulation. The immunological tolerance is typically brought about by a mechanism of active suppression.

The invention provides a kit that may be used to carry out the therapeutic treatment, which kit comprises

(a) a first formulation comprising a compound in a dosage form for administration by an oromucosal route to induce immunological

tolerance to the drug, and

(b) a second formulation comprising the drug in a dosage form for administration to effect the therapeutic treatment.

Furthermore, the invention provides:

- use of an immunogenic drug for the manufacture of a second formulation for administration to effect therapeutic treatment of a disease of a human or animal, wherein the human or animal has become immunologically tolerant to the drug through administration by an oromucosal route of a first formulation comprising a compound that induces the immunological tolerance; and

- use of a compound for the manufacture of a first formulation for oromucosal administration to a human or animal to induce immunological tolerance to an immunogenic drug, wherein the human or animal is also administered a second formulation comprising the drug to effect a therapeutic treatment.

DETAILED DESCRIPTION OF THE INVENTION

The immunogenic drug

The immunogenic drug used in the invention may be any drug that, when administered to a subject, induces an immune response that reduces the pharmacokinetics and/or effectiveness of the drug. The invention is particularly applicable to drugs that are administered in repeat doses over a period of time because the problem of rejection of such drugs by the immune system is greater than with drugs that are only administered once. The invention is also particularly applicable to expensive drugs because it reduces the amount of drug that needs to be administered to effect the therapeutic treatment; in conventional treatment regimes immunogenic drugs often have to be administered in large doses to allow for the fact

that the pharmacokinetics and/or effectiveness of the drug are reduced by the immune system.

The drug used in the invention is generally a peptide drug. The term "peptide" as used herein means any molecule comprising a chain of six or more amino acids and includes full length proteins and polypeptides. The peptide may, for example, be from 10 to 500 amino acids, from 20 to 300 amino acids or from 50 to 200 amino acids.

The peptide drugs that may be used in the invention include:

interferons - e.g. IFNα, IFNβ, IFNγ, IFNω and IFNτ
cytokines - e.g. interleukin-1 (IL-1), IL-2, IL-4, IL-5, IL-12, IL-15, IL18 and TGF-β
growth factors - e.g. granulocyte-macrophage stimulating factor (GM-CSF), granulocyte stimulating factor (G-CSF), erythropoietin (EPO) and IL-3
soluble receptors - e.g. tumour necrosis factor alpha soluble receptor (TNFα SR) and IL-1 SR
antibodies - e.g. anti-IFNα, anti-IFNβ, anti-TNFα, anti-IFNα R, anti-IFNγ R and soluble receptor - IgG fusion proteins.

Interferons are anti-viral proteins produced by cells such as leukocytes and fibroblasts in response to viral infection. Interferons such as IFNα are useful not only in the treatment of viral diseases, such as chronic hepatitis B and C, herpes and influenza, but also in the treatment of a variety of hematological malignancies including hairy cell leukemia, chronic myelogenous leukemia, low grade lymphomas, cutaneous T-cell lymphomas, and solid tumors such as renal cell carcinoma, melanoma, carcinoid tumors, and AIDS-related Kaposi's sarcoma. Recombinant IFN β is also useful for the treatment of remitting relapsing multiple sclerosis. Thus, the invention can be applied to the treatment of viral diseases, cancers and multiple sclerosis.

Recombinant human IL-2 has been used extensively for the treatment of a number of neoplastic diseases including renal cell carcinoma (Rozenberg et al., 1989, Ann, Surg., 210:474) and malignant melanoma (Rozenberg et al., 1987, New Engl. J. Med., 316:889), and to a lesser extent for the treatment of certain virus diseases including chronic hepatitis B (Kakumu et al., 1988, Hepatology, 8:487-492). More recently intermittent systemic administration of recombinant IL-2 has been used as a means of increasing CD4+ T cell proliferation in AIDS patients treated with 3'-azido-3'-deoxythymidine (AZT) (Snellerl, M.C., Antiviral Res., 29:105-109).

G-CSF, GM-CSF and IL-3 are used principally to treat the neutropenia and leukopenia observed in cancer patients following chemotherapy. The invention can therefore be used to treat these conditions.

Erythropoietin is a protein, normally produced in the liver of the fetus and in the kidneys of adults, which plays a role in the regulation of the oxygen level of the blood by stimulating the proliferation and differentiation of red blood cell precursor cells to mature red blood cells. EPO can be administered to subjects for the treatment of diseases associated with inadequate red blood cell supply, e.g. anaemia and chronic renal failure. The invention can therefore be applied to the treatment of these diseases.

TNFα is a potent molecule which elicits a broad spectrum of biological responses. The release of TNFα by macrophages induces local effects that participate in protection against infection. However, TNFα can also have damaging effects; it induces septic shock when released systemically and is believed to play a role in autoimmune diseases such as rheumatoid arthritis. Thus, antibodies against TNFα and soluble TNFα receptors that bind to and sequester TNFα are useful in the treatment of septic shock and rheumatoid arthritis. A number of such antibodies and receptors are in development. for example antibody cA2 (Centocor), antibody cDP571 (Celltech), antibody D2E7 (CAT), receptor p75-Fc IgG (Immunex) and receptor p55-Fc IgG (Roche). The invention can be used to enhance the clinical

effectiveness of such antibodies and receptors in the treatment of septic shock and autoimmune diseases such as rheumatoid arthritis.

The antibodies useable in the invention are generally monoclonal, and are preferably humanised or human. However, one advantage of the invention is that it may allow the use of non-humanised antibodies that cannot normally be used for therapy because they are rejected by the immune system. In humanised antibodies, the complementarity determining regions (CDRs) of a non-human antibody of a therapeutically useful specificity are grafted into the framework of a human antibody. This produces an antibody which has the therapeutically useful specificity but appears less foreign to the immune system than the donor non-human antibody. Such humanised antibodies go some way to overcoming the problem of rejection by the immune system but, as mentioned above, they do not eliminate the problem but merely slow down the formation of anti-idiotype antibodies.

The drug may be a fusion protein. Such proteins are particularly prone to being rejected by the immune system because the process of fusing two proteins generates new epitopes, for example in the sequence around the fusion point. Examples of fusion proteins useable in the invention are chimeric antibodies; bispecific antibodies; humanised antibodies; and antibodies linked to other peptides, for example antibodies linked to soluble receptors (e.g. TNFα soluble receptors).

The drug may be a nucleic acid (e.g. DNA) encoding a therapeutic peptide. The nucleic acid is expressed in the subject, thereby producing the peptide. In this way, the invention can be applied to diseases treatable by gene therapy, for example cystic fibrosis and Gaucher disease.

The nucleic acid is generally delivered in a vector. It is desirable to induce immunological tolerance not only to the therapeutic peptide encoded by the nucleic acid but also to the nucleic acid and/or the vector. In order to achieve this double tolerance, the compound administered in the first formulation may be a nucleic acid

(e.g. DNA) encoding a peptide that induces tolerance. Alternatively, the compound administered in the first formulation may be a peptide, in which case immunological tolerance is generated against the peptide only (not the nucleic acid).

Vector systems for administering nucleic acids to effect gene therapy are well-known in the art, and these known systems can be used in the invention. The nucleic acids used in the invention can, for example, be administered in naked DNA vectors, viral vectors or cellular vectors. The vectors comprise a sequence encoding the therapeutic peptide linked to a control sequence which is capable of providing for the transcription of the coding sequence.

Examples of viral vecors useable in the invention include retroviruses, adenoviruses and adeno-associated virus vectors. Examples of cellular vectors include microorganisms that colonise the intestines, such as *Salmonella* and *E.coli* bacteria. Uptake of naked DNA constructs by mammalian cells is enhanced by several known transfection techniques, for example those including the use of transfection agents. Examples of such agents include cationic agents (for example calcium phosphate and DEAE-dextran) and lipofectants (for example lipofectam™ and transfectam™).

Thus, administration of the first formulation induces tolerance to the vector to prevent its elimination by the immune system and loss of the transducing gene. Furthermore, administration of the first formulation induces tolerence to recombinant protein expression systems for the production of peptides such as IL-2, IFNα and clotting factor IX, thereby allowing therapeutic or curative levels of the peptide to be attained *in vivo*.

The compound in the first formulation does not have to be the same as the drug in the second formulation in order for the compound to produce immunological tolerance against the drug. For example, oral treatment with one interferon may induce immunological tolerance not only to the orally administered interferon but

also to another interferon. Thus, the compound in the first formulation may be the same or different to the drug in the second formulation.

The compound in the first formulation will normally be structurally related to the drug in the second formulation. In the case of peptides, the drug in the second formulation may be a full length protein and the compound in the first formulation may comprise a partial sequence of the protein containing one or more epitopes of the protein. A peptide in the first formulation may, for example, have at least 50%, at least 70%, at least 90%, at least 95% or at least 98% sequence identity to the peptide in the second formulation or to a part of the sequence of the peptide in the second formulation. The two peptides may differ by, for example, from 1 to 100, from 1 to 50, from 1 to 20 or from 1 to 10 amino acids.

The compound in the first formulation and the drug in the second formulation will normally be from the same class of compounds. Thus, when the drug is a cytokine (e.g. an interleukin) the compound will normally be a cytokine (e.g. an interleukin), when the drug is a type I or type II interferon the compound will normally be a type I or type II interferon respectively, when the drug is a growth factor the compound will normally be a growth factor, when the drug is a soluble receptor the compound will normally be a soluble receptor and when the drug is an antibody the compound will normally be an antibody.

The treatment regimen

As described above, the basic treatment regimen according to the invention comprises administering a first formulation to a subject by an oromucosal route, e.g. orally comprising a compound in a dosage form which induces immunological tolerance to the drug, and administering a second formulation to the subject by a parenteral route comprising the drug in a dosage form that effects the therapeutic treatment.

The dose of compound and drug administered in the first and second formulations will also vary according to factors such as the nature of the drug and the disease to be treated. The dose of the compound in the first formulation is such that it induces immunological tolerance against the drug to be administered in the second formulation. The tolerance is preferably achieved through a mechanism of active suppression. The dose of drug administered in the second formulation is such that it is therapeutically effective. A typical dose of the compound in the first formulation may be from 0.1 μg to 10 mg, for example from 1 μg to 5 mg or from 5 μg to 1 mg. A typical dose of the drug in the second formulation may be from 1 μg to 100 mg, for example from 5 μg to 100 mg.

Various test methods can be used to optimise the conditions for a particular course of treatment. When the invention is applied clinically, it may be desirable to confirm that immunological tolerance has been induced by the first formulation before administration of the second formulation. This could be achieved by carrying out a skin test with the drug; an absent or low reaction in such a test is indicative that immunological tolerance has been induced. It would be desirable to carry out a control skin test before administration of the first formulation to obtain a result for comparison with the result of the test carried out after administration of the first formulation; any reduction in the reaction obtained in the later test compared to the reaction obtained in the test prior to administration of the first formulation is indicative of immunological tolerance.

It is possible to confirm the induction of immunological tolerance in laboratory and clinical experiments by (i) determining the level of antibody against the drug (e.g. IgG or IgM) in the blood serum of a test subject after administration of the first and second formulations and (ii) determining the level of antibody against the drug in the blood serum of a control subject who has been administered the second but not the first formulation. Any reduction in the level of antibody in the serum of the test subject compared to the control subject is indicative of immunological tolerance. The antibody response may be measured by conventional

The dosages and timing of the doses will to a certain extent be up to the discretion of the physician, but can be optimised for each drug. The optimum doses and timing for each drug will vary according to factors such as the disease to be treated, the route of administration and the condition and weight of the patient.

The first formulation is generally administered prior to administration of the second formulation, but some benefit of immunological tolerance may be obtained if the two formulations are administered substantially simultaneously. Typically, the first formulation is administered from 0 to 28 days, from 1 to 14 or from 4 to 7 days prior to the second formulation. In any event, the relative timing of the administration of the two formulations is such that the subject exhibits oral tolerance to the drug in the second formulation.

Multiple doses of the first and/or second formulation may be administered. The first formulation may, for example, be administered in from 1 to 14, from 1 to 7 or from 1 to 4 doses. It may be administered once a day for a number of days before administration of the second formulation. For example, the first formulation may be administered every day for from 1 to 4, 1 to 7 or 1 to 14 days. The final dose of the first formulation may typically be given the day before the second formulation is administered, but it is also envisaged that there could be a delay before administration of the second formulation to allow immunological tolerance to build up; this delay could, for example, be from 1 to 7 or 1 to 4 days.

The number of doses of the second formulation and the frequency of the doses will vary according to factors such as the nature of the drug, the disease to be treated and how quickly the subject recovers from the disease. In some cases, only a single dose of the second formulation will be given, but the invention is particularly suited to treatment of diseases where it is necessary to give multiple doses of drug. For example, the drug may be administered in multiple doses over a period of from 2 to 56 days or 4 to 28 days. The number of doses may, for example, be from 1 to 14, from 1 to 7 or from 1 to 3.

techniques, such as ELISA.

The first formulation is administered by an oromucosal route, i.e. the oral route or the nasal route. The second formulation may be administered by any route such that a therapeutic effect is obtained. The formulation is generally administered parenterally or systemically. The exact route of administration chosen will again depend on factors such as the nature of the drug and the disease, but peripheral routes such as the intravenous, subcutaneous, intramuscular, intrathecal and intraperitoneal routes are appropriate.

The first and second formulations typically contain a pharmaceutically acceptable carrier or diluent. The first formulation may take the form of a tablet, capsule, liquid solution, slow release formulation, liposome, aerosol or spray for oral administration. The second formulation typically takes the form of a solution, for example in isotonic saline.

EXAMPLES

The following Examples serve to illustrate the invention.

Brief Description of the Drawings

Figure 1 shows a standard curve for anti-human IFNα2 in an ELISA for quantification of anti-IFNα antibodies in the sera of test animals.

Figure 2 shows the IgM antibody response to recombinant human IFNα2 in Swiss mice at 4 days after i.v. injection of the IFNα2. The mice had been pre-treated with BSA alone or with various doses of the IFNα2 in BSA by the oromucosal route.

Figure 3 shows the IgM antibody response to recombinant human IFNβ in Swiss mice at 0, 7, 14, 21 and 28 days after i.v. injection of the IFNβ. The mice had been pre-treated by the oromucosal route with 10^3, 10^4, 10^5, or 10^6 IU of the IFNβ. The first bar in each group of five is for excipient alone, the second is for 10^3 IU, the third is for 10^4 IU, the fourth is for 10^5 IU and the fifth is for 10^6 IU.

Figure 4 shows the IgM antibody response to recombinant human IFNα2 in BALB/C mice at 21 days after subcutaneous injection of the IFN. The mice had been pretreated with various doses of IFNα2 by the oromucosal route once a day for four days prior to the injection.

Figure 5 shows the IgG antibody response to recombinant human IFNα2 in BALB/c mice at 0, 7, 14 and 28 days after subcutaneous injection of 3×10^5 IU of human IFNα2 without adjuvant three times a week for four weeks. The mice had been pretreated once a day for 7 days by the oromucosal route with PBS alone (first base), BSA 100 μg/ml in PBS (second bar), 5×10^3 IU of IFNα2 diluted in BSA/PBS (third bar), 10^6 (fourth bar) or 10^7 (fifth bar) IU of IFNα2 alone.

Figure 6 shows the IgG antibody response of BALB/c mice to recombinant GM-CSF 21 days after subcutaneous injection of 3 μg of GM-CSF without adjuvant three times per week for four weeks. The mice had been pre-treated by the oromucosal route with various doses of recombinant GM-CSF (0, 1μg, 5μg or 10 μg) or injected with 1.0 μg of human serum albumin alone in PBS once a day for 7 days.

EXAMPLE 1

This Example illustrates the induction of oral tolerance to interferons.

MATERIALS AND METHODS

Pharmaceutical Components

Recombinant human IFNα2

Carrier-free recombinant human interferon α2 (Roferon™ A) at a concentration of 9×10^6 IU/ml and a specific activity of 2×10^8 IU/mg protein was obtained from Hoffmann-la-Roche, Basel, Switzerland. The interferon was in aqueous isotonic solution in an acetate buffer at pH 5.0.

Recombinant human IFNβ

Pure recombinant human IFNβ (specific activity 2×10^8 IU/mg) was obtained from Toray Industries Inc., Kamakura, Japan at a concentration of 440 μg/ml in isotonic phosphate buffer at pH 7.0.

Bovine serum albumin/phosphate buffered saline excipient.

Bovine serum albumin fraction V RIA grade, immunoglobulin free (cat no. A7888, Sigma, MO, USA) was dissolved at a final concentration of 100 μg/ml in phosphate buffered saline (pH 7.4) and sterile filtered (0.2 μ).

ELISA for the quantification of anti-IFNα antibodies

Briefly, microtiter plates (NUNC Maxisorb™ cat. no. 442406) were coated with 100 µl per well of a 2 µg/ml solution of Roferon™ IFNα2 in 0.1 M solution of $NaHCO_3$ at pH 8.5 overnight at 4°C. The plates were then washed twice with 200 µl of PBS, and treated for 2 hrs with 100 µl per well of 2% gelatine (Sigma, cat. no. G7765) in PBS. The plates were then washed 3 times with 0.05% Tween 20™ (Biorad) in PBS, dried and stored at 4°C until use. Each serum sample was then tested at 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} starting dilutions. Serial two fold dilutions of each starting dilution in 2% gelatine in PBS were incubated for 1 hr at 20°C (100 µl per well). The plates were then washed 4x with 0.05% Tween™ in PBS (200 µl per well). The plates were then treated with 100 µl of a 1:5,000 dilution in 2% gelatine in PBS of either a peroxidase conjugated goat anti-human IgG or a peroxidase conjugated goat-anti-human IgM antibody (Interchim, cat. no. 115-035-062) and 115-035-020 respectively). The plates were then washed 4 X with 200 µl per well of 0.05% Tween™ in PBS. One hundred µl per well of a 400 µl/ml solution of o-phenylenediamine dihydrochloride (Sigma, cat. no. P1526) in 44 mM citrate buffer pH 5.0 together with 0.03% H_2O_2 was added per well. The plates were then incubated for 30 minutes at 20°C and 50 µl per well of 0.5 µl H_2SO_4 was added. The optical density was then determined at 490 nM in a Metentech™ 960 spectrophotometer.

Results are expressed in arbitrary units determined from the reciprocal of the dilution obtained from a standard curve of serial two fold dilutions (1:100 to 1:12,800) of a specific anti-interferon α2 monoclonal antibody (mAb I 18) (Figure 1).

Experimental Procedure

Groups of 6-week old BALB/c male mice from a specific pathogen-free breeding colony were treated with various doses of recombinant human IFNα or IFNβ or with excipient alone once a day for 4 or 7 days by the oromucosal route in a volume of 10 µl of BSA/PBS excipient. Animals were then injected intravenously or

subcutaneously with 200 µl of interferon as indicated in the figure descriptions above. Two animals from each treatment group were then sacrificed at pre-determined time points, e.g. 0, 7, 14, 21 and 28 days, and whole blood was recovered, pooled, and the serum was assayed for the presence of specific antibodies to the interferon.

Oromucosal administration

Previous experiments had shown that the application of 5 µl of crystal violet to each nostril of a normal adult mouse using a P20 Eppendorf micropipette resulted in an almost immediate distribution of the dye over the whole surface of the oropharyngeal cavity. Staining of the oropharyngeal cavity was still apparent some 30 minutes after application of the dye. These results were confirmed by using ^{125}I-labelled recombinant human IFN-α1-8 applied in the same manner. The same method of administration was employed to effect oromucosal administration in all the studies which are described below.

RESULTS

In an initial experiment, groups of Swiss mice were treated by the oromucosal route with various doses of recombinant human IFNα or IFNβ in BSA/PBS, or with excipient alone for 4 days prior to intravenous injection of 9×10^5 IU of IFNα or IFNβ respectively.

Pretreatment of animals with increasing doses of recombinant IFNα2 by the oromucosal route resulted in a corresponding dose dependent reduction in the IgM antibody response following iv injection of recombinant human IFNα2 (Figure 2).

Pretreatment of animals with IFNβ by the oromucosal route also resulted in a reduced IgM antibody response following intravenous injection of recombinant IFNβ (Figure 3). A similar degree of inhibition of IgM antibody production was observed, however, when animals were pretreated by the oromucosal route with 10^3, 10^4, 10^5 or

10^6 IU of recombinant IFNβ determined at 1, 2, 3 or 4 weeks (Figure 3).

In a second experiment, groups of 6 week old Balb/c mice were treated by the oromucosal route with various doses of recombinant human IFNα in BSA/PBS, or with excipient alone once a day for 4 days prior to intravenous injection of 9×10^5 IU of IFNα.

Pretreatment of animals with increasing doses of recombinant IFNα2 by the oromucosal route resulted in a corresponding dose dependent reduction in the IgM antibody response following subcutaneous injection of recombinant human IFNα2 (Figure 4).

Referring to Figure 5, mice were pretreated once a day for 7 days by the oromucosal route with $5x10^3$, 10^6 or 10^7 IU of recombinant IFNα2 prior to subcutaneous injection of $3x10^5$ IU of recombinant IFNα2 without adjuvant three times a week for four weeks. The IgG antibody response to recombinant human IFNα2 in the mice was then determined using a specific ELISA for anti-IFNα2 IgG at 0, 7, 14 and 28 days after subcutaneous injection of recombinant human IFNα2. As shown by Figure 5, treatment of animals with IFNα2 by the oral route induced a dose dependent inhibition of antibody production upon subsequent subcutaneous administration of IFNα2 with complete suppression of antibody production being observed in animals treated orally with the highest dose of interferon tested (10^7IU).

These results suggest that the oral administration of a protein based drug prior to parenteral administration is an effective means of reducing the antibody response (IgM or IgG) to the drug by the induction of active tolerance, and should find wide application for the repeated use of protein-based drugs for the treatment of chronic diseases.

EXAMPLE 2

This Example illustrates the induction of oral tolerance to recombinant GM-CSF.

Source of recombinant GM-CSF

Pure recombinant human GM-CSF (Leucomax™) specific activity 1 x 10^7/mg protein, at a concentration of 4.4 x 10^6 IU/ml and containing 1.0 mg/ml of human serum albumin, as manufactured by Schering-Plough was obtained from Novartis France.

Experimental Procedure

Groups BALB/c mice were pretreated once a day for 7 days by the oromucosal route with 1.0, 5.0 or 10.0 μg of recombinant human GM-CSF prior to subcutaneous injection of 3.0 μg of recombinant GM-CSF without adjuvant three times a week for four weeks. The IgG antibody response to recombinant human GM-CSF in the mice was then determined using a specific ELISA for anti-GM-CSF IgG at, for example, 21 days after subcutaneous injection of recombinant human GM-CSF. The results at 21 days after subcutaneous injection shown in Figure 6 are expressed as the mean value for 6 animals per time point.

CLAIMS

1. A kit for therapeutic treatment of a subject with an immunogenic drug, which kit comprises

   (a) a first formulation comprising a compound in a dosage form for administration by an oromucosal route to induce immunological tolerance to the drug, and

   (b) a second formulation comprising the drug in a dosage form for administration to effect the therapeutic treatment.

2. A method for therapeutic treatment of a subject with an immunogenic drug, which method comprises

   (a) administering to the subject by an oromucosal route a first formulation comprising a compound in a dosage form which induces immunological tolerance to the drug, and

   (b) administering to the subject a second formulation comprising the drug in a dosage form that effects the therapeutic treatment.

3. Use of an immunogenic drug for the manufacture of a second formulation for administration to effect therapeutic treatment of a disease of a human or animal, wherein the human or animal has become immunologically tolerant to the drug through administration by an oromucosal route of a first formulation comprising a compound that induces the immunological tolerance.

4. Use of a compound for the manufacture of a first formulation for oromucosal administration to a human or animal to induce immunological tolerance to an immunogenic drug, wherein the human or animal is also administered a second formulation comprising the drug to effect a therapeutic treatment.

5. A kit, method or use according to any one of the preceding claims, wherein the compound in the first formulation is the same as the drug in the second formulation.

6. A kit, method or use according to any one of the preceding claims, wherein the drug is a peptide.

7. A kit, method or use according to claim 6, wherein the drug is a fusion protein.

8. A kit, method or use according to any one of claims 1 to 6, wherein the drug is an interferon, a cytokine, a growth factor, a soluble receptor or an antibody.

9. A kit, method or use according to claim 8, wherein the drug is an interferon.

10. A kit, method or use according to claim 6, wherein the drug is interferon alpha (IFNα), IFNβ, IFNγ, IFNω, IFNτ, interleukin-1 (IL-1), IL-2, IL-4, IL-5, IL-12, IL-15, IL-18, tranforming growth factor beta (TGFβ), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte-stimulating factor (G-CSF), IL-3, erythropoietin (EPO), soluble tumour necrosis factor alpha receptor (TNFα SR), soluble interleukin-1 receptor (IL-1 SR), anti-IFNα antibody, anti-IFNβ antibody, anti-TNFα antibody, anti-IFNα R antibody, anti-IFNγ R antibody, or a soluble receptor-IgG fusion protein.

11. A kit, method or use according to any one of claims 1 to 5, wherein the drug is a DNA molecule.

12. A kit, method or use according to any one of the preceding claims, wherein the first formulation is in a dosage form for administration by the oral route.

13. A kit, method or use according to any one of the preceding claims, wherein the second formulation is in a dosage form for administration by a systemic route.

14. A kit, method or use according to any one of claims 1 to 11, wherein the second formulation is in a dosage form for intravenous, subcutaneous, intramuscular, intrathecal or intraperitoneal administration.

15. A kit, method or use according to any one of the preceding claims, wherein the first formulation is in the form of a tablet, capsule, liquid solution, slow release formulation, liposome, aerosol or spray.

16. A kit, method or use according to any one of the preceding claims, wherein the first formulation comprises the compound in a dose of 1 μg to 5 mg.

17. A kit, method or use according to any one of the preceding claims, wherein the second formulation comprises the drug in a dose of 5 μg to 100 mg.

18. A kit, method or use according to any one of the preceding claims, wherein the immunological tolerance induced by the first formulation is by a mechanism of active suppression.

19. A method or use according to any one of claims 2 to 18, wherein the first formulation is administered from 1 to 14 days prior to administration of the second formulation.

20. A method or use according to any one of claims 2 to 19, wherein the first formulation is administered from 4 to 7 days prior to the second formulation.

21. A formulation comprising a pharmaceutically acceptable carrier or diluent and a drug as defined in any one of claims 7 to 11 in a dosage form for administration by the oral route to induce immunological tolerance.

Fig.1.

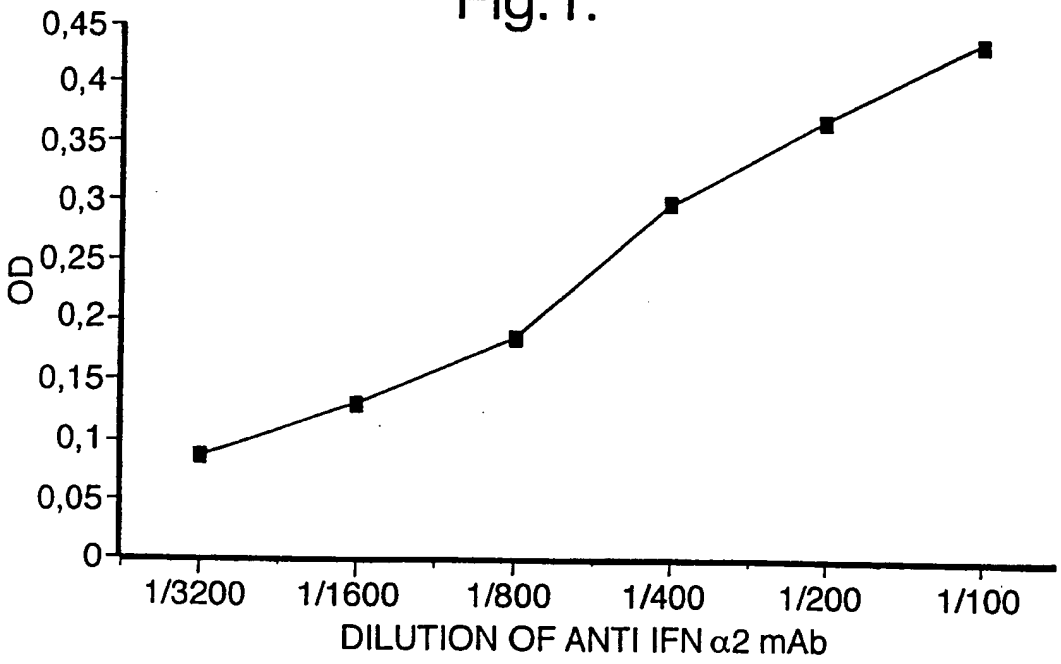

0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
OD
1/3200
1/1600
1/800
1/400
1/200
1/100
DILUTION OF ANTI IFN α2 mAb

Fig.2.

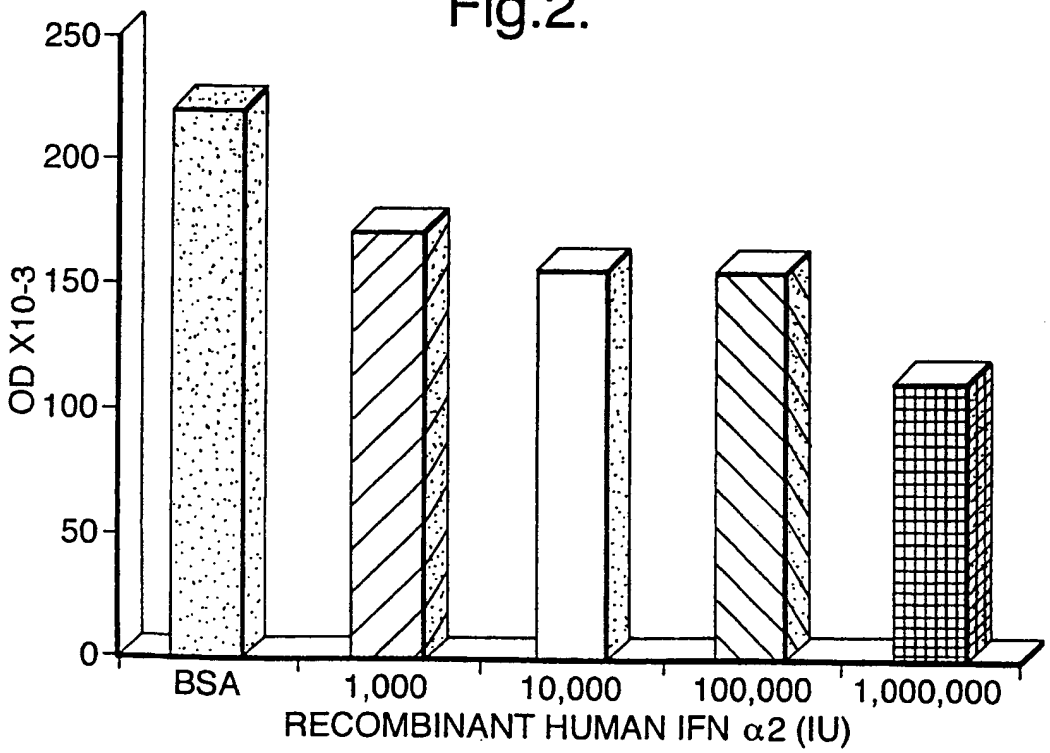
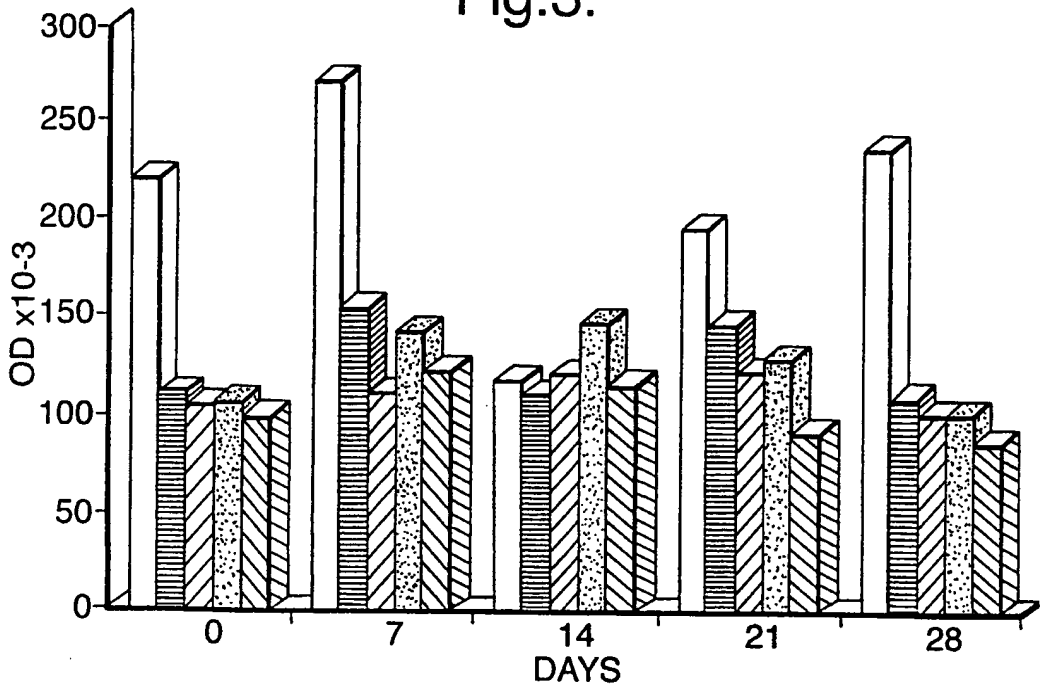

250
200
150
100
50
0
OD X10-3
BSA
1,000
10,000
100,000
1,000,000
RECOMBINANT HUMAN IFN α2 (IU)

Fig.3.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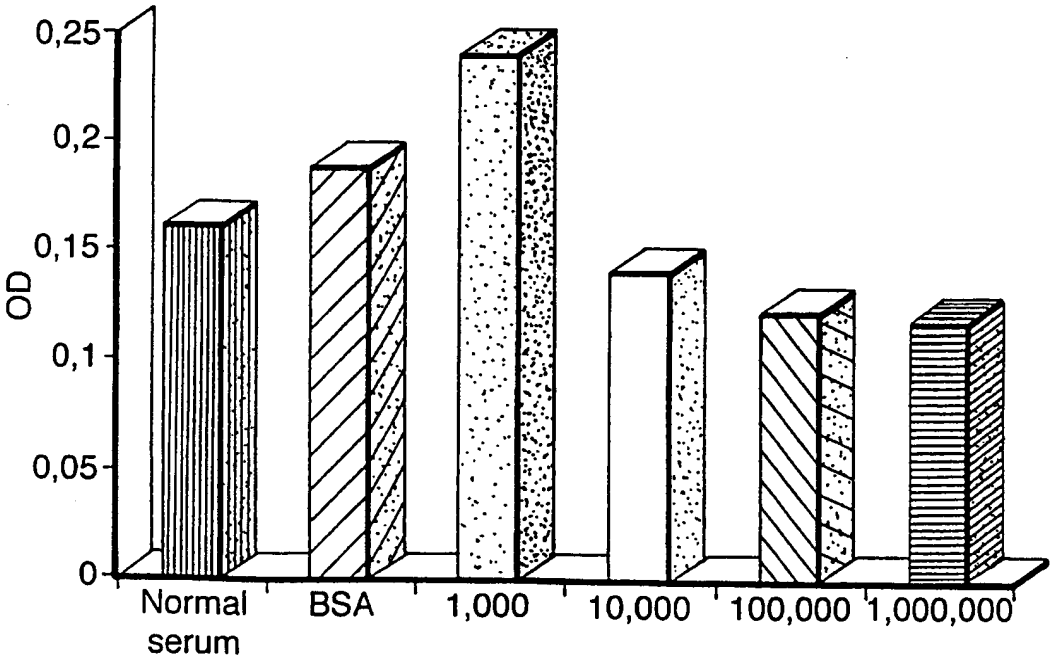
200
150
100
50
0
OD x10-3
0
7
14
21
28
DAYS

Fig.4.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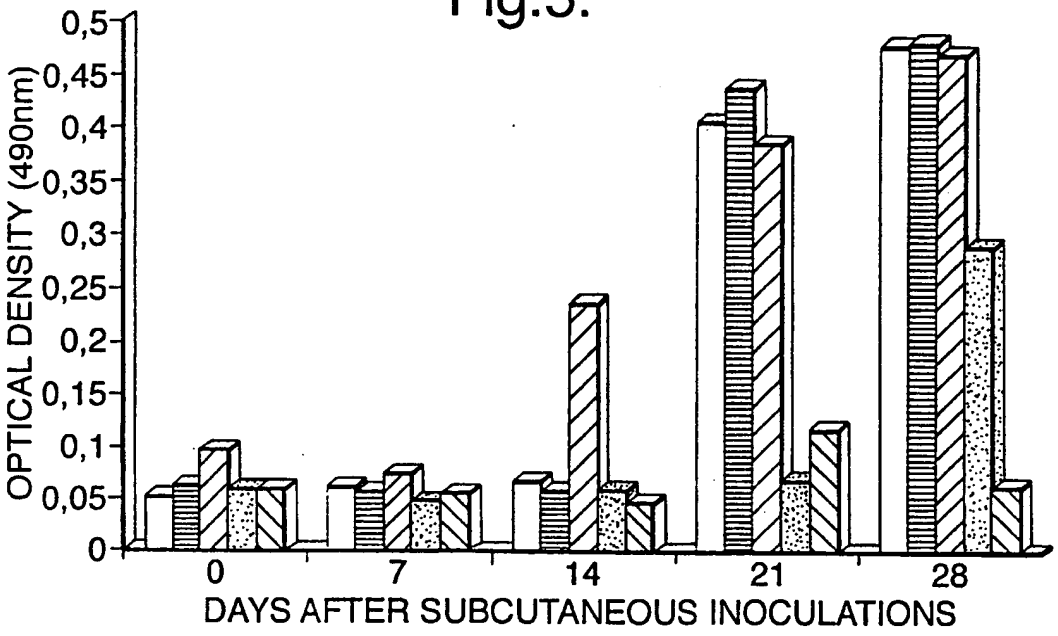
0,05
0
OD
Normal serum
BSA
1,000
10,000
100,000
1,000,000

Fig.5.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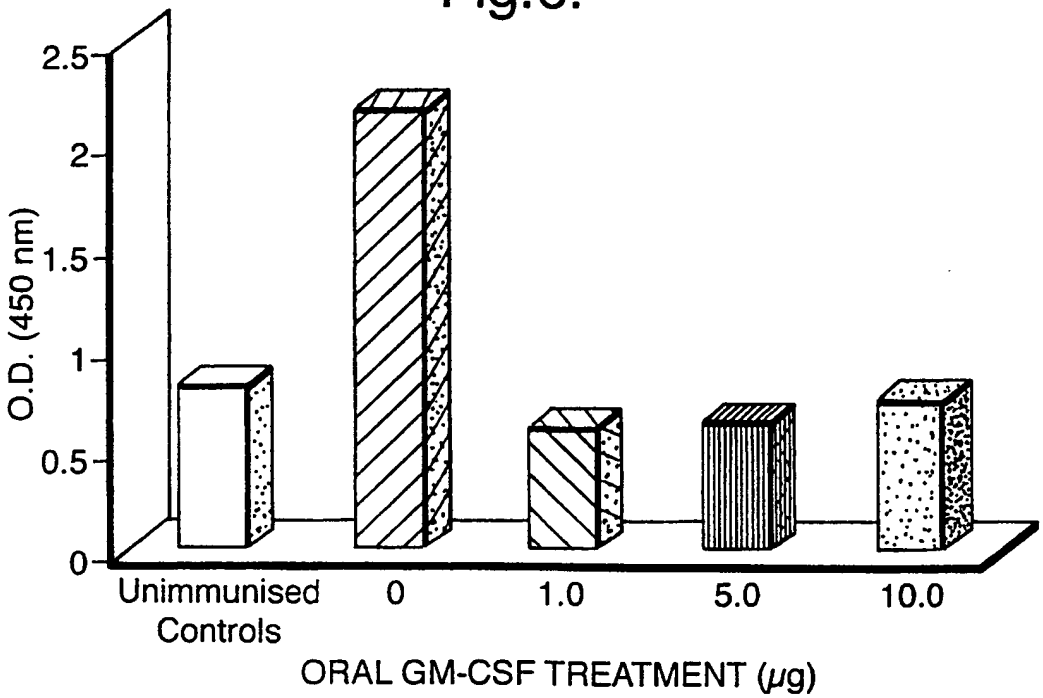
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
0
7
14
21
28
DAYS AFTER SUBCUTANEOUS INOCULATIONS

Fig.6.


O.D. (450 nm)
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2
1.5
1
0.5
0
Unimmunised Controls
0
1.0
5.0
10.0
ORAL GM-CSF TREATMENT (μg)